

## UNPUBLISHED PRELIMINARY DATA

From: Bruce L. Welch, Director, Laboratory Population Ecology,  
Department of Biology, College of William and Mary,  
Williamsburg, Virginia

To: Office of Grants and Research Contracts, Attention Code SC,  
National Aeronautics and Space Administration, Washington  
25, D. C.

Via: Dr. W. Melville Jones, Dean of Faculty, College of William  
and Mary, Williamsburg, Virginia

Subj: Semi-annual Status Report of Research Performed on the  
project "Psychophysiological Effects of Prolonged Con-  
finement in Small Groups and in Isolation", financed as  
project NSG-567-B under the NASA Research Grant NSG-567  
to the College of William and Mary.

1. Twenty-five preprints of each of two papers which have been  
submitted for publication are enclosed as part of this report.  
As these papers have not yet been accepted for publication,  
they are not for general distribution.

Submitted to Science: "Long-Term Isolation 'Stress': A Dif-  
ferent View".

Although long-term isolation (22 weeks) of mice may be  
a stressful experience, it does not cause adrenalcortical  
hypertrophy. Instead, it causes hypotrophy of the  
adrenal cortex, as compared with adrenals of mice kept  
in groups of 2-3; and mice kept in groups of 2-3 have  
smaller adrenals than mice kept in groups of 9-14. It  
is questioned if adrenalcortical hypertrophy is a neces-  
sary effect of stress, or if, more precisely, it is caused  
by increased levels of stimulation.

Submitted to Journal of Neurochemistry: "Brain Noradrenaline  
in White Mice Chronically Aggregated and Isolated for One Week".

The brains of white mice isolated for one week contain  
more noradrenaline than those of mice kept in a group  
of twenty individuals for the same period of time. The  
increased excitability of chronically isolated animals  
may be related to a build-up of brain noradrenaline.

2. Analysis of data on three experiments is proceeding. These  
are:

An experiment to determine the effect of long-term isolation  
(22 weeks) upon the brain content of noradrenaline and  
upon adrenal medullary catecholamines. Attention to the lat-  
ter effect is part of a larger ongoing study of adrenal medul-  
lary adaptation to different sustained demands for catechol-  
amine secretion.

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An experiment to study the effect of chronic (5-week) aggregation of mice upon d-amphetamine toxicity. Amphetamine in this experiment is used as a crude indicator of differences in endogenous metabolism of catecholamines.



An experiment to study the effect of long-term confinement in isolation, in pairs, and in groups of four individuals upon adrenal size, adrenal medullary catecholamines, and brain noradrenaline under conditions where physical contact is not permitted. This experiment seeks to eliminate temperature as a casual factor in the effects of aggregation.

3. Much of the time since receipt of funds three months ago, has been spent assembling staff and equipment. Certain major items of equipment for radioactive work are only now arriving. A building is being renovated to provide seventeen rooms for animal care and housing, but it is not yet complete. It has not yet been possible to commence extensive long-term experiments or to begin precise studies of metabolic pathways.

Sincerely yours,

*Bruce L. Welch*

Bruce L. Welch, Director  
Laboratory of Population  
Ecology



Bruce L. Welch, Page 1

Long-Term Isolation "Stress": A Different View

A 12377

Abstract: Male white swiss mice isolated for a long period (22 weeks) become hyperexcitable and aggressive, but they do not develop enlarged adrenals. Adrenal weights are significantly smaller in mice housed individually than in groups of 2-3, and in these than in groups of 9-13. This is true not only for the mean weights, but for the minima and maxima as well. These differences may be accentuated with time. Although long-term isolation may be considered "stressful," in that it is less than an optimum environmental situation and elicits abnormal behavioral and physiological responses, it probably does not cause adrenalcortical hyperfunction. Possibly increased adrenalcortical activity may be more precisely related to an increased level of stimulation than to "stress" per se.

Author

In a recent issue of Science, Hatch et al (1) reported that rats subjected to 13 weeks of "isolation stress" exhibited enhanced nervousness and excitability, developed caudal dermatitis, were more susceptible to isoproterenol toxicity, and developed differences in the weight of certain organs as compared with rats kept in community cages. Particular attention was directed to the fact that the isolated rats had "heavier adrenals" and this was taken to indicate that long-term isolation had caused "an endocrinopathy with hyperfunction of the adrenal cortex," which played a central role in the effects observed. The experimental data presented in their paper does not support the conclusions drawn. The opposite result, reduction in adrenal size, has been obtained in mice kept in long-term isolation.

Hatch et al reported only adrenal weight relative to body weight; no actual adrenal weights and no actual body weights were reported. Inasmuch as the differences between relative adrenal weights of isolated and community-housed rats reported in Table I were quite small, i.e. 0.013 gm vs. 0.011 gm for males (reported non-significant) and 0.030 vs. 0.024 for females ( $p=.01$ ), it occurred to me that these apparent differences might actually be attributable to simply a smaller body weight in the isolated animals. Deprived of the opportunity to huddle to conserve body heat, small mammals often have lower body weights in isolation than in groups, particularly if the ambient temperature is low, although the opposite is

frequently the case when the ambient temperature is high (2).

In response to a letter of inquiry which I wrote concerning absolute adrenal weights and the possibility of using covariance analysis to remove body weight effect, Dr. Hatch replied "...there was no significant difference in absolute adrenal weights in either sex (10 per group), but a significant increase in the adrenalcortical width of the isolated females was found. When adrenal weight is plotted against body weight, the isolated and community rats give two distinct curves. This is owing to the smaller body weight of the isolated rats." In view of this, her continued statement that, "The two curves could be said to represent two dose response lines since we consider the isolated stress to be the result of a chronic endogenous hypersecretion of ACTH," seems hardly justified. The greater adrenalcortical width of the isolated females (which was not reported in the published paper) may possibly be related to the effects of population density upon fecundity of female rats (3) and to changes in the adrenal cortex known to come about in female rats with changes in reproductive condition (4).

The quoted abstract report of a similar experiment by Weltman et al (5) in which albino mice were isolated for 14 weeks indicated that there was no significant increase in relative adrenal weight of isolated mice, in spite of the fact that their body weight was slightly smaller than that of mice in pairs. This would imply that the isolated mice actually had somewhat smaller adrenals. Rats

isolated for a period of 11 weeks have recently been reported to have smaller pituitaries and slightly smaller regenerated adrenals than rats kept in groups of 3 for the same period of time, a result which the authors interpreted to indicate greater ACTH secretion in the grouped situation (6). Unfortunately these differences may also be somewhat illusory, since they too were expressed as relative weights and the body weights of the grouped rats were significantly less than those of the isolates. Again, actual body weights and actual adrenal weights were not given, and the magnitude of the real difference cannot be computed.

When male chickens were isolated for 29.5 weeks followed by three weeks of (a) continued isolation, (b) penning in groups of 5 for four hours daily or (c) penning continuously in groups of 5, the adrenals were significantly smaller in the isolated birds than in those continuously grouped while the adrenal weights of the intermittently grouped birds were intermediate in size (7).

A number of relatively short duration experiments have compared the adrenals of isolated and variously grouped laboratory mice. One paper reported no significant difference between adrenal weights of white mice isolated and variously grouped for 1 week, the adrenals of the isolates being slightly heavier in absolute weight and slightly smaller in relative weights than those of grouped mice (8). The failure for a significant difference to develop in this instance may have been due, at least in part, to the short term

of the experiment. Suggestive evidence that the difference between adrenal weights of grouped and isolated mice is accentuated with time is given later in this paper. The same author reported that placing male white mice, which had been isolated for 3 weeks, in groups of 4 for one hour each day resulted in an 80% eosinopenia throughout the 8 day experimental period (9).

In several short term experiments of 1-3 weeks duration, both actual and relative adrenal weights of isolated mice were generally smaller than those of mice variously grouped in numbers of up to 30 mice per group (10). Histological studies of the adrenals were made in some of these experiments and these supported the indications of the differences in weight (11). In similar experiments adrenal ascorbic acid was found to be lower in the adrenals of grouped than isolated white mice (12). Notably, in these experiments the change in adrenalcortical size was relatively sensitive as well as directional, there being a general tendency (with exceptions) for each increase in group size to cause an increase in size of the adrenal cortex. This same pattern of adrenalcortical response has been observed in the present relatively long-term experiment.

Male white Swiss mice (DUB/ICR, Dublin Laboratory Animals, Dublin, Virginia), 5-6 weeks of age were maintained individually (n=14), in groups of 3 (n=12) and in groups of 14 (n=28 mice) for a period of 22 weeks. Cages 10" x 7" x 7" with sides and backs of metal, and top, bottom and front of wire were used; they were elevated to permit good air circulation. Purina laboratory feed

was maintained in superabundance over the floor of the cages at all times; four water-bottles were on each group cage. Handling and cleaning was uniform.

Table 1 shows that the mean actual adrenal weight increased from 1.889 mg in the isolates, to 2.018 mg in the 3-groups, to 2.479 mg in the 14-groups. If adrenal weights were expressed relative to body weight, the difference between the isolates and the 14-groups would be accentuated by the slightly smaller body weight in the latter. Because the variance as well as the mean increased with increasing group size and is significantly different for the 3 treatment conditions (Bartlett's test  $p=.001+++$ ,  $\chi^2 = 38.2$  df 2, where  $\chi^2 .001=13.82$ ), the weights were compared non-parametrically and by the use of weighted means. Thus compared, the difference in adrenal weights is highly significant; by analysis of variance utilizing weighted means (13),  $p=.001+++$  ( $F=81.62$ , df 2, 21, where  $F .001=9.77$ ). The general nature of the trend for mean adrenal weight to increase with increased density is emphasized by the fact that not only the mean weight, but also the minimum and the maximum adrenal weights increased with increasing density (Table 1).

The fact that isolation reduces (or that grouping increases) the variability of physiological response as indicated by adrenal size should be of interest to those planning experiments. That this variability is caused by factors of social interaction is

indicated by the fact that the largest adrenal weight and the smallest adrenal weight in the 14-groups belonged, respectively, to the worst scarred (obvious social subordinate) and to a completely unscarred sleek individual (an obvious dominant). Some theoretical implications of this phenomena of sociophysiological differentiation have been considered separately (14).

In Table 2 the adrenal weight of isolated mice is expressed as a percentage of the adrenal weight of grouped mice. The percentages obtained in the present relatively long-term experiment are compared with percentages similarly derived from data obtained in several short-term experiments of 1-3 weeks where adrenals of isolated mice and mice in comparable-sized groups to those employed in this experiment were available for comparison. Although male white mice of the same general age were used in each of these experiments and were of the same breed in all experiments conducted by me, the experiments were conducted at different times and in different locations and are comparable, therefore, only in a general way. Nevertheless, they do indicate that the well-established tendency for grouped white mice to have larger adrenals than individually-housed white mice in short-term experiments of 1-3 weeks is not likely reversed in longer term experiments. Indeed, they suggest that these differences may be accentuated as the duration of the experiment increases. This tendency is more pronounced for the larger groups than for the smaller. This apparent enhancement with time of the differences between the adrenal

weight of isolated and grouped mice probably reflects the intensified effect of long-term grouping rather than the cumulative effect of long-term isolation. In support of this interpretation is the fact that Bullough found the maximum cross-sectional area of the adrenal cortex of male white mice housed in groups of 20 to increase from 57.1 to 68.1, to 75.8 arbitrary planimetered units after 1, 2, and 3 weeks, respectively, in the experimental grouping condition (16).

There is no question that isolation and social deprivation may induce abnormal behavioral and physiological changes (15). But if isolation is a "stressor" and it certainly is not the optimal environmental situation for an organism, it is probably not manifest by increased adrenalcortical function. We must inquire, therefore, if increased adrenalcortical function is actually a generalized indication of stress. Possibly not. All of the experiments upon which this concept is based have employed "stresses" which had the common effect of causing pain, annoyance, or otherwise increasing the level of stimulation which the experimental animals experienced (17). Increased adrenocortical function may simply be indicative of an increased level of stimulation.

In humans, social isolation, sensory deprivation and monotonously repeated stimuli cause depression and slowing of the mental processes (19). College students isolated for 96 hours in bed wearing translucent goggles, which admitted diffuse light but

prevented patterned vision, and cotton gloves and cardboard cuffs, which limited tactual stimuli, showed a progressive slowing of EEG activity throughout the isolation period, and changes persisted for 3½ hours after emerging from isolation (20). Zubek and co-workers reported a progressive decrease of alpha frequencies on EEG traces during 7-14 days in isolation. The tracings were still abnormally low a week later, and long-lasting motivational losses were observed (21).

A report of studies of perceptual deprivation by D. O. Hebb's group at McGill University, in which men were kept abed in isolation up to 6 days wearing translucent goggles, etc. said, "...one may safely conclude that the corticoid excretion is not consistently or at any time markedly increased with this type of sustained perceptual deprivation," in spite of the fact that some of the subjects were overtly distressed and complained of hallucinations (22). Lilly wrote of his studies of sensory deprivation in immersion tanks that "as the levels of stimuli were lowered closer to zero, the positive, more blissful, enjoyable states appeared" (23).

Several studies of natural populations of wild mammals have shown the adrenal to hypertrophy and other evidence of increased adrenalcortical activity to become apparent as population density increased, and/or for such differences to exist between two populations of different sizes (24).

Figure 1 diagrammatically shows one model for the apparent adrenalcortical response to the environmental level of stimulations. Inasmuch as social stratification is known to exist within groups of mice such that the social subordinates have larger adrenal cortices than the social dominants (25), and, presumably, experience higher levels of stimulation, appropriate arrows have been included to show this distinction.

Probably there is an optimum level of social stimulation somewhere between overpopulation and isolation. Deviation from this optimum social environment in either direction may be a "stress" in that the organism is required to adapt to the new situation. But it may be that environmental change evokes increased adrenalcortical activity only if it causes the level of environmental stimulation to be increased. Long-term isolation presumably reduces the level of sensory input from the environment and apparently does not have this effect (26).

Bruce L. Welch, Director  
Laboratory of Population Ecology  
Department of Biology  
College of William and Mary  
Williamsburg, Virginia

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Fig. 1. Model for adrenalcortical response to the environmental level of stimulation (ELS). Stress is represented as a deviation in either direction from a central optimum. Social stimulation is represented as the major (though not the only) affecting stimulus. Separate arrows for social dominants and social subordinates acknowledge stratal differences within all ELS of groups or population density. The progressively diverging and thickening arrows represent an increasing variability.

Table 1. Adrenal weight of male white mice isolated and grouped for 22 weeks.

Size Group	N	Body Wt. (gm.)	Paired Adrenal Wt. (mg.)			Variance
			Mean	Minimum	Maximum	
Isolate	22	39.37	1.89	1.49	2.65	.0818
3(2-3)*	10	39.87	2.02	1.58	2.96	.1923
14(9-13)*	19	38.46	2.48	1.89	3.67	.2488

\*Initial numbers reduced by fighting as indicated in parentheses.

Table 2. Apparent accentuation of the difference between adrenal weights of isolated and grouped male white swiss mice with increased time in treatment conditions.

Experiment	Duration Expt. (wks.)	Larger groups compared with isolates				Smaller groups compared with isolates			
		Size	Isolate adrenal wt.		Group	Size	Isolate adrenal wt.		Group
			Actual wt.	as percent of grouped Rel. wt.			Actual wt.	as percent of grouped Rel. wt.	
Present Experiment	22	14*	76.2	74.4		3#	93.6	94.8	
Welch (in preparation)	3								
Expt. I		16	86.8	80.9	4		103.5	89.8	
		8	104.1	95.3	2		98.8	100.4	
Expt. II		8	66.1	72.2	4		71.6	75.0	
					2		72.7	80.7	
Welch and Klopfer, 1961 (10)	2	16	85.9	82.5	4		89.5	97.1	
		8	82.6	78.1	2		96.0	95.7	
Christian, 1955 (10)	1	16	92.1	88.8	4		95.4	90.9	
Am. J. Physiol. 182		8	92.4	90.9					
Am. J. Physiol. 181		16	92.3	86.2	4		96.3	89.1	
Christian, 1959 (3)	1	8	95.2	87.0					
Table 1, large cage		16	88.4	95.1					
Christian, 1959 (18)	1					4-5	91.7		

\* Reduced to 9-13 by fighting

# Reduced to 2-3 by fighting

BRAIN NORADRENALINE IN WHITE MICE  
CHRONICALLY AGGREGATED AND ISOLATED FOR ONE WEEK\*

Bruce L. Welch

Laboratory of Population Ecology

Department of Biology

The College of William and Mary

Williamsburg, Virginia

*Index as NIMH<sup>14</sup> MHO...*

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Moore (1963) demonstrated that aggregation potentiates the reduction of brain noradrenaline by d-amphetamine in mice. Vogt (1954) emphasized that the many drugs tested which cause a fall in hypothalamic noradrenaline have in common only that they stimulate <sup>peripheral</sup> sympathetic centers. Using the difference in catecholamine content of the innervated and denervated adrenals of cats as an index of sympathetic activity caused by administered drugs, including amphetamine, she found a high correlation between the depletion of <sup>adrenal</sup> medullary amines and loss of hypothalamic noradrenaline (Vogt, 1962).

The application of electric shock through a wire grid upon which rats were standing, a procedure which presumably caused increased sympathetic activity, lowered brain stem noradrenaline 17% in 5 minutes, and 37% in 45 minutes (Maynert and Levi, 1964). Shocks similarly applied to guinea pigs lowered their brain noradrenaline 38% (Paulson and Hess, 1963). When rats were forced to swim to exhaustion their brain noradrenaline was markedly lowered (Barchas and Freedman, 1963; Freedman, 1963).

Welch and Welch (1964b) offer evidence that the basal level of secretion of adrenal medullary catecholamines may be higher in grouped than in isolated white mice and in large groups than small. Collectively, the foregoing studies suggest that the stimulus of grouping may cause brain noradrenaline levels to be lowered, and that under the condition of relative stimulus deprivation existing in social isolation, brain noradrenaline stores may be increased above normal amounts.

This paper reports an experiment in which mice isolated one week had brain noradrenaline stores 27% greater than mice maintained 20 per group for the same period of time.

12378

Author

## MATERIALS AND METHODS

Forty male DUB/ICR white Swiss mice 2-3 months of age, were placed in individual enamel mouse pans 8 1/8"x 13 3/8"x 4 3/4" upon receipt from the supplier (Dublin Laboratory Animals, Dublin, Virginia). After one week 20 mice were placed in a single cage and the remaining 20 were continued in individual cages. Purina laboratory food was scattered liberally over the floor of the pan such that it was equally available to all animals, and it was kept in superabundance at all times. Six water bottles were maintained on top of the group pan. All animal pans were changed twice during the course of the week by the same person.

At the end of one week in experimental groupings, the mice were killed by decapitation. They were killed between 10:00 a.m. and 2:00 p.m., alternating with groups of 4 mice from each treatment condition in order to avoid any bias due to circadian rhythm. The brains of the 4 mice killed in each group were pooled, and all later manipulations were performed with paired samples of 4 brains, one from each of the treatment conditions. The brains were frozen on dry ice as removed from the animals, and analyses for catecholamines were conducted 24 hours later. The brains were weighed, homogenized in 0.4 normal perchloric acid at 0°C, adjusted to pH 6.1 with 0.5 normal potassium carbonate, and centrifuged at 10,000 rpm for 10 minutes to sediment protein and potassium perchlorate. The catecholamines were adsorbed on a 2 cm column of Amberlite IRC-50 ion exchange resin and buffered at pH 6.1 with ammonium acetate. They were eluted from the column with 30 ml of 0.5 normal acetic acid following a distilled water wash. The eluate was concentrated to small volume in vacuo, an analysis for noradrenaline was conducted by the method of Cohen and Goldenberg (1957).

## RESULTS

There was more noradrenaline in the brains of isolated mice than in the brains of mice living 20 per group. The amount in the brains of the isolated mice was greater in each of the paired samples representing the two treatment conditions. The mean values for noradrenaline, as shown in Table 1, were .26  $\mu\text{g}/\text{gm}$  brain for the grouped mice and .33  $\mu\text{g}/\text{gm}$  brain for the isolates. By analysis of variance, this difference is highly significant ( $p$  .001).

A small amount of fighting occurred among the grouped mice during the first few hours, but during the remainder of the experimental period fighting was very rare. The isolated mice were noticeably more excitable than those living in the group.

## DISCUSSION

White mice isolated for one week have higher levels of brain noradrenaline than white mice housed 20 per group for the same period of time.

A feedback mechanism is probably operative whereby catecholamines released from the adrenal medulla act upon the ascending reticular activating system to cause a release of brain noradrenaline (Vogt, 1962, 1959, 1954). This is a likely mechanism for the enhancement of amphetamine toxicity by aggregation, and for the accentuated lowering of brain noradrenaline by d-amphetamine in aggregated mice (Moore, 1963).

The lowering of threshold to d-amphetamine toxicity caused by electric shock (Askew, 1962), which also lowers brain noradrenaline (Maynort and Levi, 1964; Paulson and Hess, 1963), is prevented by adrenalectomy and by the administration of phenoxybenzamine, an adrenergic blocking agent (Weiss et.al., 1961). The work of Swinyard's group suggest that acute aggregation may cause an enhanced peripheral sympathetic activity (Bonson et.al., 1963; Swinyard et.al. 1963; Swinyard et.al., 1961).

In studies of chronic grouping, in which graded group sizes of 1, 2, 4, 8, 16, 20 and 30 mice per group were employed, Welch and Welch (1964b) found changes in adrenal medullary catecholamine content indicative of increased basal secretion with increased group size. Studies of d-amphetamine toxicity in mice chronically grouped for five weeks gave substantiating results (Welch, in preparation).

It is interesting to speculate that the changed excitability and aggressiveness of mice upon chronic isolation or chronic grouping may be related to a change in the level of brain noradrenaline. Isolation is well known to make animals more aggressive and excitable (Balazs et.al., 1962; Scott and Froderickson, 1951; Bronson and Eleftheriou, 1963), and the tranquilizing drugs which deplete stores of brain noradrenaline reduce this aggressive behavior (Kollar and Umbreit, 1956; Barnes, 1960; Janssen et.al., 1960;

Yen et.al. 1959), while inhibitors of the enzymes which catabolize catecholamines make the animals hyperexcitable and enhance aggressive behavior (Everett and Wiegand, 1962).

C3H mice living in isolation became so sensitive to environmental stimuli that they convulsed during routine procedures such as weighing, cage-changing, feeding, or even opening the cage door for inspection. Such convulsions were markedly reduced by housing the mice in pairs and were virtually eliminated by keeping them in groups of 20 (King et.al., 1955). Brain noradrenaline is lowered by 35% in hedgehogs during hibernation (Uuspaa, 1963), a period during which the threshold of excitability is lowered. In the experiments of Welch and Welch (1964b), in which the level of secretion of catecholamines apparently increased with increased group size, the incidence of fighting decreased in graded manner from the largest to the smallest sized group, there being only one-tenth as many fights per unit time per individual in groups of 20 mice as in pairs (Welch, in preparation). It is anticipated that experiments in progress may show a graded lowering of brain noradrenaline from isolated through variously sized groups of aggregated mice. However, a parallel between brain noradrenaline and aggressiveness does not necessarily mean that they are causally related.

In these analyses, no correction was made for 3,4-dihydroxyphenylethylamine (dopamine). Hence, up to 8% of the noradrenaline values quoted could be attributed to dopamine. However, to the extent that the change in level of excitability which occurs with isolation or grouping may be related to brain catecholamines, it is probably more closely related to noradrenaline than to dopamine. Baird and Lewis (1963) reported a reduction of noradrenaline, but not of dopamine, in rat brain by d-amphetamine; and Lal et. al. (1963) found the time-course of protection from amphetamine toxicity in grouped mice provided by alpha methyl dihydroxyphenylalanine and alpha methyl metatyrosine to follow the same time course as that of the effects of these agents on brain noradrenaline.

A number of studies have demonstrated that the chronic effects of intensified social stimulation associated with increased group size in laboratory rodents and increased population density in laboratory and field populations of various mammals causes increased activity of the pituitary-adrenocortical axis (Christian, 1961, 1959; Welch, 1962; Welch and Klopfer, 1961). The reflex release of hypothalamic noradrenaline by the action of peripheral catecholamines upon the reticular activating system may be a link in the chain of events leading to activation of the pituitary-adrenocortical axis following increased autonomic stimulation. In emotional response adrenaline presumably activates the hypothalamus via the ascending reticular formation and causes corticotrophin releasing factor to be released. This polypeptide is carried to the anterior pituitary where it releases ACTH, thus causing the adrenal cortex to increase its output of corticoids (Vogt, 1954; Werner, 1962). The lowering of brain biogenic amines following a forced exhaustion swim or following forced activity in a rotating wheel occurs in rats even if they have been hypophysectomized (Barchas and Freedman, 1963), indicating that this response is probably independent of the action of the adrenal cortex. However, apparently only a pituitary which has functional connection with the brain can develop ACTH and maintain the adrenal cortex (Scharrer and Scharrer, 1963). In anencephali, in which the brain is absent while the adenohypophysis is well developed, the adrenals are abnormally small (Moeri, 1961).

The possibility that the metabolism of catecholamines and/or their catabolites in the brain may be modified in a categorically predictable manner by the social environment in which an animal lives (Welch and Welch, 1964a) may have important implications for our understanding of the relationship between environment and mental health.

## SUMMARY

The noradrenaline content of the brain of male white Swiss mice grouped 20 per cage for one week was lower than that of mice housed in isolation for the same period of time, i.e., .26  $\mu\text{g}/\text{gm}$  brain v.s. .33  $\mu\text{g}/\text{gm}$  brain ( $p < .001$ ). The possible relation of these findings to aggressive behavior and excitability; a possible mechanism by which this change in brain noradrenaline may be effected; and the possible relation of these changes to other physiological changes occurring in individuals with change in group size or population density are discussed.

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Table 1. Noradrenaline in Brain of Grouped and Isolated Mice

Replicate sample runs (4 brains each)	Noradrenaline (ugm/gm brain)	
	Grouped 20/cage	Isolated
1	.27	.32
2	.25	.35
3	.28	.32
4	.26	.31
5	.23	.34
Mean	.26 $\pm$ .01	.33 $\pm$ .01

Significance = .001 ( $F_{1,8} = 37.6$ )